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(21) International Application Number: PCT/CA94/00158 (22) International Filing Date: 11 March 1994 (11.03.94) (30) Priority Data: 08/032,045 16 March 1993 (16.03.93) US (71) Applicant (for all designated States except US): WESTAIM TECHNOLOGIES INC. [CA/CA]; 10101 - 114 Street, Fort Saskatchewan, Alberta T8L 2P2 (CA). (72) Inventor; and (75) Inventor/Applicant (for US only): BURRELL, Robert, Edward [CA/CA]; 112 Village Downs, Sherwood Park, Alberta T8A 4L6 (CA). (74) Agent: OGILVIE AND COMPANY; #1400 Metropolitan Place, 10303 Jasper Avenue, Edmonton, Alberta T5J 3N6 (CA).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>With amended claims.</i>
(54) Title: ENHANCED MICROTITRE PLATE AND IMMUNOASSAYS CONDUCTED THEREIN (57) Abstract A microtitre plate containing a plurality of wells for conducting immunoassays wherein one or more of the sides, bottom and lid walls of each well are formed with a reflective, metallized surface. The surface may be an inner coating on the walls of clear or opaque plates, or an outer coating on the walls of clear plates. Alternatively, the plate may be formed, for example, by stamping from a reflective metal material. Improved sensitivity in photometric detection results from photon emitting immunoassays conducted in the plate.		

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1 **"ENHANCED MICROTITRE PLATE AND IMMUNOASSAYS CONDUCTED**
2 **THEREIN"**

3 **FIELD OF THE INVENTION**

4 This invention relates to microtitre plates and
5 immunogenic assays conducted therein.

6 **BACKGROUND OF THE INVENTION**

7 Microtitre plates are moulded plastic plates or
8 trays having a plurality of depressions or wells in which
9 chemical and biological tests are carried out. Immunogenic
10 assays involving an antibody-antigen reaction to detect an
11 antibody or antigen in biological materials such as serum
12 are most commonly performed in microtitre plates.
13 Colorimetric immunoassays are widely used, the most common
14 type being an ELISA (enzyme-linked immunosorbent assay)
15 method. This technique is an enzyme immunoassay used for
16 the detection of antibodies or antigens. Antigens are
17 immobilized on the surfaces of the wells of the microtitre
18 plates, following which, the sample to be assayed for
19 particular antibodies, typically serum, is added to the
20 wells in specific assay dilutions. This step is followed by
21 the immuno detection of the antibody/antigen complexes,
22 usually by photometrically measuring an enzymatically
23 labelled colour-forming immune complex. In colorimetric
24 immunoassays, a source of incident light is directed onto or
25 through the assay reagents (typically in a clear multi-well
26 microtitre plate). A portion of the radiant energy of the
27 incident beam is absorbed by a component the assay. The
28 light which is transmitted from the assay is detected by a
29 suitable photometer to provide a measurement of the
30 concentration of a component of the assay. Colorimetric
31 immunoassays are photon absorbing assays.

32 Other photometric assay techniques performed in
33 microtitre plates are termed photon emitting immunoassays.
34 These include chemiluminescent, bioluminescent and
35 fluorescent immunoassays. In these assays photons are
36 emitted, for example as a result of the action of an enzyme,
37 or through the use of particular photon emitting labels.

1 Emitted photons are detected by suitable photometric devices
2 such as luminometers or fluorometers.

3 Immunoassays are typically conducted in microtitre
4 plates. Such plates are typically made of a light
5 transmitting plastic polymer such as polystyrene or
6 polyvinylchloride. It is usually important that the
7 microtitre plate be formed from a light transmitting plastic
8 since reading of the assay results is typically done through
9 the contents in the wells, particularly for colorimetric
10 immunoassays.

11 In the case of photon emitting immunoassays, the
12 microtitre plates may be made of opaque plastic, such as
13 black or white polystyrene, in order to reduce "cross-talk"
14 in photometrically reading the results from well to well
15 (i.e. to reduce interference caused by stray photons).
16 While cross-talk may be lessened by such opaque microtitre
17 plates, the sensitivity of the results is lessened. For
18 instance, black plates absorb some of the photons being
19 emitted from the plates while white plates simply scatter
20 the photons.

21 Japanese Patent 61-215947 teaches that the optical
22 interference in colorimetric immunoassays carried out in
23 transparent microtitre plates can be reduced by constructing
24 wells optically independently with an opaque layer on the
25 well walls.

26 An immunoassay technique of greater
27 sensitivity often resorted to is a radioimmunoassay (RIA).
28 However, this technique is costly both in time and equipment
29 needs as it involves the use of radioactive materials.
30 These materials require special handling and licenses from
31 the time they are ordered until they are safely stored at a
32 radioactive waste site. However, even with the drawbacks,
33 the high degree of sensitivity provided by this technique
34 for some assays (i.e. where the amount of material being
35 assayed is very small or where the volume of sample is very
36 small) makes it an indispensable diagnostic tool. If these
37 levels of sensitivity could be reached by a non-isotopic

1 method, then costs could be dramatically lowered and a much
2 safer work environment could be achieved.

3 There is a need for a microtitre plate which
4 provides for improved sensitivity in photometric immunoassay
5 techniques, particularly for photon emitting immunoassays
6 such as, chemiluminescent, bioluminescent and fluorescent
7 immunoassays.

8 SUMMARY OF THE INVENTION

9 This invention overcomes the above mentioned
10 difficulties by providing a microtitre plate with a
11 multiplicity of wells or depressions with walls that are
12 highly reflective. The plates may be formed with a
13 reflective metal, ceramic or semiconductor coating on one or
14 both of the side or bottom walls of the wells.
15 Alternatively, the plates may be formed from reflective
16 metal. Still another alternative is to place a clear
17 microtitre plate inside a reflective plate to read the assay
18 results. Preferred reflective metals, ceramics or semi-
19 conductors for coatings include (in a highly reflective
20 form) aluminum, tin, magnesium, zinc, cadmium, indium,
21 transition metals such as silver, chromium, gold, platinum
22 and nickel, silicon, germanium, silica and alumina or alloys
23 containing at least one of these metals. The plates may
24 also be stamped from a highly reflective metal, for example,
25 from bright aluminum foil.

26 The invention also provides an improved method of
27 conducting a photon emitting immunoassay in the reflective
28 plates. Enhanced sensitivity results from the use of the
29 reflective plates.

30 Broadly stated the invention provides a microtitre
31 plate comprising a plate member having top and bottom
32 surfaces, the top surface defining a plurality of spaced,
33 upwardly opening wells, each of said wells having side
34 and/or bottom walls with inner and outer surface, and one or
35 both of the side and bottom walls of said wells providing a
36 reflective, metallized surface.

1 The invention also provides an improved method of
2 conducting a photon emitting immunoassay in a multi-well
3 microtitre plate, wherein an antigen-antibody complex is
4 formed in the wells and photons emitted from the wells are
5 detected by a photometric device. The improvement comprises
6 conducting the immunoassay in a microtitre plate having a
7 reflective metallized surface on one or both of the side or
8 bottom walls of the wells to reflect photons emitted from
9 the wells into the photometric device.

10 It should be understood that the term "wells", as
11 used herein and in the claims, in association with
12 microtitre plates, is meant to include both depressions
13 having only a bottom wall (ex. shallow, round bottom
14 depressions), and generally cylindrical wells, having both
15 side and bottom walls. Generally cylindrical wells
16 typically have round, flat or conical bottom walls. The
17 side walls are typically vertical, but could vary, for
18 example as conical or fluted side walls.

19 It should also be understood that the term "walls"
20 as used herein and in the claims is meant to include the
21 inner or outer surfaces of the wells, regardless of the well
22 shape. Thus, the term wall, when used in association with
23 a depression shaped well, refers to its rounded surface,
24 although the depression does not in fact possess discrete
25 side and bottom walls.

26 The phrase "reflective, metallized", as used
27 herein and in the claims, is meant to include highly
28 reflective surfaces produced from shiny metallic materials,
29 for instance, aluminum, tin, transition metals, ceramics and
30 semiconductors (all in a highly reflective form), as
31 distinct from surfaces that are merely light scattering due
32 to glassy or glossy properties.

33 **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

34 The microtitre plates in accordance with the
35 present invention are preferably produced by coating
36 standard multi-well microtitre plates with a reflective,

1 metallized coating on at least a portion of the inner or
2 outer side or bottom walls of the plates. Alternatively,
3 the plates according to this invention are formed by
4 stamping the wells into a reflective metal, such as bright
5 aluminum foil. Still another alternative is to place a
6 standard clear microtitre plate containing the assay inside
7 a second microtitre dish having a reflective metallized
8 coating on the inner side and/or bottom wells.

9 The reflective metallized coatings are preferably
10 metal coatings because they are highly reflective and
11 inexpensive to apply. However, ceramic or semiconductor
12 materials may be applied as highly reflective coatings in
13 assay applications demanding an inert surface or a surface
14 with particular immobilization qualities.

15 If a clear, transparent microtitre plate is used,
16 the coating may be provided on the inner side and/or bottom
17 walls of the plates. Alternatively, with a transparent
18 microtitre plate, the outer side and/or bottom walls of the
19 plates are coated. If an opaque, coloured plate is used,
20 the coating is provided on the inner side and/or bottom
21 walls. The particular areas coated will vary with the
22 particular assay technique and photometric plate reading
23 equipment which is to be used. For instance, for bottom
24 read plates, the bottoms of the wells may be left uncoated.
25 Bottom read plates may include a reflective lid or plate
26 cover for use in the actual reading equipment. Top read
27 plates, which are most commonly used, are preferably coated
28 on both the bottom and side walls to maximize the advantages
29 of the reflective coating.

30 The walls of the microtitre plates may be shaped
31 to maximize the utility of the reflective coating. For
32 example, the walls may be curved (ex. rounded or parabolic)
33 or faceted to maximize upward reflections of photons into
34 the photometric detecting equipment.

35 State of the art coating techniques for example
36 physical vapour deposition, chemical vapour deposition or
37 electroless deposition, may be used. Physical vapour

1 deposition techniques include sputtering, magnetron
2 sputtering, vacuum deposition and ion plating.

3 The coating is applied to a thickness such that
4 the walls of the plate are no longer transparent to light,
5 but are highly reflective of light.

6 Microtitre plates which are coated on the outside
7 walls with the metallized reflective coating are preferred
8 to minimize immobilization problems encountered in the assay
9 techniques. An outer reflective coating preserves the inner
10 wall surface (typically polystyrene) for which most
11 immunoassay techniques have been designed. If the inner
12 walls are coated with the metallized reflective coating, the
13 assay technique may be altered to overcome any
14 immobilization problems. Alternatively, a clear, inner
15 coating of a plastic such as polystyrene may be provided on
16 the walls themselves to avoid toxicity or immobilization
17 problems with the metal coating. Reflective ceramic or
18 semiconductor coatings may also be advantageously used to
19 overcome toxicity or immobilization problems.

20 Shiny metals can be used for the reflective
21 metallized coating, for example, aluminum, tin, magnesium,
22 zinc, cadmium, indium, transition metals such as silver,
23 nickel, gold, platinum and chromium or alloys of these or
24 other metals. Thin reflective coatings of ceramics, such as
25 alumina and silica, or semiconductors such as silicon and
26 germanium, may also be used. Aluminum is particularly
27 preferred since it does not interfere significantly with the
28 assay techniques. The particular coating material or alloy
29 chosen will vary with the assay technique to be performed in
30 the wells.

31 Immunoassay techniques are well known in the art.
32 The metallized reflective plates of the present invention
33 are advantageously used in colorimetric immunoassays such as
34 ELISA, to enhance the sensitivity of the readings off the
35 plates. However, the plates are particularly useful in
36 photon emitting immunoassays, including chemiluminescent,
37 bioluminescent and fluorescent immunoassays. Such assays

are described in detail in the literature, see for example, L.J. Kricka and T.J.N. Carter, Clinical and Biochemical Luminescence, Marcel Dekker, New York (1982); L.J.Kricka et al., Analytical Applications of Bioluminescence and Chemiluminescence, Academic Press, New York (1984); and N. Monji and A. Castro, Conjunction of Haptens and Macromolecules to Phycobilli Protein for Application in Florescence Immunoassay, Reviews on Immunoassay Technology, S.B.Pal (ed), Vol 1, Chapman and Hall, New York (1988).

If the plates used in photon emitting assays have a metallized reflective coating on the outer walls of the otherwise transparent plates, the assays may be conducted without alteration. However, if the coating is on the inner walls of the plate, and immobilization or toxicity problems are encountered with the metal coating, the assay technique should be modified to include a first step of coating the inner walls with an adherent plastic film. A reflective coating of ceramic or semiconductor material may also be used. These latter coatings might also be applied, in a clear form, over the reflective coating.

The invention is further illustrated by the following non-limiting examples.

Example 1

This example illustrates the preparation of a metallized microtitre plate in accordance with the present invention. A standard clear flat bottomed polystyrene microtitre plate (obtained from Corning) was coated by magnetron sputtering with aluminum metal as follows:

Magnetron Sputtering Conditions:

Equipment - Perkin Elmer 4410
Target - 99.999% Aluminum
Power - 1kW
Time - 20 min
R.P.M. - 3
Coating Thickness - 4000 Angstroms
Base Pressure - 2×10^{-7} Torr
Cathode/Substrate Distance - 65 mm

Working Gas - Argon

Working Gas Pressure - 20 mT

The plates were oriented on the substrate table such that either the insides of the individual wells and the top side of the plate or the outsides of the individual wells and the bottom side of the top of plates were metallized (line of sight coating). The coating thickness on the flat portions of the plates coated at normal incidence was 4000 Angstroms. On the inside vertical walls of the wells, the coating thickness varied from 4000 Angstroms at the top to about 500 Angstroms at the bottom (the aluminum coating was not transparent at the bottom).

Example 2

The two plates coated in accordance with the procedure set forth in Example 1 (i.e. metallized reflective coating on the inside or outside side and bottom walls of the wells) were tested both biologically and physically to demonstrate utility for immunoassays and to evaluate for reflectivity.

a) Reflectivity Tests

The reflectivity of the each of the plates was compared to the reflectivity of standard clear, white and black microtitre plates using a Nanospec AFT reflectometer. The instrument was standardized against an aluminized optically flat silicon wafer (coated under the conditions set forth in Example 1). Data was collected as a percentage of the standard. The results were as follows:

Standard	100%
Plate, metallized inner walls	98%
Plate, metallized outer walls	96%
Plate, clear polystyrene	9.0%
Plate, white polystyrene	7.5%
Plate, black polystyrene	4.0%

The metallized plates in accordance with the present invention were clearly much more reflective than the clear or opaque, coloured plates. This is in part due to the lack of scattering and absorption of light in the

1 metallized plates relative to the clear and opaque, coloured
2 plates. This demonstrates that in any photon emitting
3 assay, the metallized plates will result in a greater
4 capture of emitted photons since photons not directed at the
5 detector initially will be redirected with minimal loss,
6 even after multiple reflections within the plate.

7 b) Biological Tests

8 A unit of alkaline phosphatase enzyme was serially
9 diluted to extinction (from 10^0 to 10^9). Samples from
10 dilutions were placed in individual wells of a metallized
11 microtitre dish (coated as in Example 1, inner side and
12 bottom walls) and a normal clear polystyrene microtitre
13 dish. All wells were precoated with bovine serum albumin to
14 minimize enzyme deactivation through adsorption. The
15 microtitre dishes were then placed into a photon counting
16 camera chamber and exposed to the enzymes substrate, p-
17 nitrophenyl phosphate. Photons from the action of the
18 enzyme on the substrate were counted if they reached the
19 camera lens system. Emitted photons were counted from both
20 plates simultaneously. It was found that the metallized
21 plates resulted in the detection of 10 times more photons
22 than did the non-metallized plate. This suggests that
23 luminescent/florescent types of assays will be at least 10
24 times as sensitive or require 10 times less sample if the
25 metallized plate is used.

26 All publications mentioned in this specification
27 are indicative of the level of skill of those skilled in the
28 art to which this invention pertains. All publications are
29 herein incorporated by reference to the same extent as if
30 each individual publication was specifically and
31 individually indicated to be incorporated by reference.

32 Although the foregoing invention has been
33 described in some detail by way of illustration and example
34 for purposes of clarity of understanding, it will be obvious
35 that certain changes and modifications may be practised
36 within the scope of the appended claims.

CLAIMS:

1. A microtitre plate comprising:
a plate member having top and bottom surfaces, the top surface defining a plurality of spaced, upwardly opening wells, each of the wells having side and/or bottom walls with inner and outer surfaces; and
one or both of the side and bottom walls of the wells providing a reflective, metallized surface.
2. The microtitre plate as set forth in claim 1, wherein the side and/or bottom walls of the wells are formed from a reflective metallized material or are provided with a reflective metal coating on the inner or outer surfaces of the well walls.
3. The microtitre plate as set forth in claim 1, wherein the side or bottom walls of the wells are formed from a clear material and a reflective metallized coating is provided on the inner or outer surfaces of the well walls.
4. The microtitre plate as set forth in claim 1, wherein the side or bottom walls of the wells are formed from an opaque material and a reflective metallized coating is provided on the inner surfaces of the well walls.
5. The microtitre plate as set forth in claim 2, wherein the reflective metallized coating is selected from the group consisting of reflective aluminum, tin, magnesium, zinc, cadmium, indium, transition metals, ceramics and semiconductors, or alloys containing at least one of these metals.
6. The microtitre plate as set forth in claim 2, wherein the metal material or coating is reflective aluminum.
7. The microtitre plate as set forth in claim 3, wherein the coating is reflective aluminum.
8. The microtitre plate as set forth in claim 4, wherein the coating is reflective aluminum.

1 9. A bottom read microtitre plate as set forth in
2 claim 1, comprising:

3 a plate member having top and bottom surfaces, the
4 top surface defining a plurality of spaced, upwardly opening
5 wells, each of the wells having side and bottom walls with
6 inner and outer surfaces;

7 a lid member having top and bottom surfaces
8 covering the upwardly opening wells;

9 one or both of the side walls of the wells and the
10 lid member providing a reflective, metallized surface; and
11 the bottom walls being substantially transparent.

12 10. The microtitre plate as set forth in claim 9,
13 wherein the side walls and lid member are formed from a
14 reflective metal material or are provided with a reflective
15 metallized coating selected from the group consisting of
16 reflective aluminum, tin, magnesium, zinc, cadmium, indium,
17 transition metals, ceramics and semiconductors, or alloys
18 containing at least one of these metals.

19 11. The microtitre plate as set forth in claim 10,
20 wherein the metal material or coating is reflective
21 aluminum.

22 12. In a method of conducting a photon emitting
23 immunoassay in a multi-well microtitre plate, wherein an
24 antigen-antibody complex is formed in the wells and photons
25 emitted from the wells are detected by a photometric device,
26 the improvement comprising:

27 conducting the immunoassay in a microtitre
28 plate having a reflective metallized surface on one or both
29 of the side or bottom walls of the wells to reflect photons
30 emitted from the wells into the photometric device.

31 13. The method as set forth in claim 12, wherein the
32 reflective surface is a reflective metallized coating on the
33 inner or outer surfaces of the well walls, said coating
34 being selected from the group consisting of reflective
35 aluminum, tin, magnesium, zinc, cadmium, indium, transition
36 metals, ceramics and semiconductors.

- 1 14. The method as set forth in claim 13, wherein the
2 reflective surface is an aluminum reflective surface on the
3 inner or outer surfaces of the well walls.

AMENDED CLAIMS

[received by the International Bureau on 22 August 1994 (22.08.94),
original claims 1-4 amended; claims 9-11 deleted;
claims 12-14 amended and renumbered as claims 9-11; other claims unchanged (2 pages)]

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1. A microtitre plate comprising:

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a plate member having top and bottom surfaces, the top surface defining a plurality of spaced, upwardly opening wells, each of the wells having side and/or bottom walls with inner and outer surfaces; and

the bottom walls, and optionally the side walls, of the wells providing a reflective, metallized surface on the inner surface of the walls.

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2. The microtitre plate as set forth in claim 1, wherein the wells are formed from a reflective metallized material or are provided with a reflective metal coating on the inner surface of the well walls.

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3. The microtitre plate as set forth in claim 1, wherein the wells are formed from a clear material and a reflective metallized coating is provided on the inner surface of the well walls.

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4. The microtitre plate as set forth in claim 1, wherein the wells are formed from an opaque material and a reflective metallized coating is provided on the inner surface of the well walls.

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5. The microtitre plate as set forth in claim 2, wherein the reflective metallized coating is selected from the group consisting of reflective aluminum, tin, magnesium, zinc, cadmium, indium, transition metals, ceramics and semiconductors, or alloys containing at least one of these metals.

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6. The microtitre plate as set forth in claim 2, wherein the metal material or coating is reflective aluminum.

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7. The microtitre plate as set forth in claim 3, wherein the coating is reflective aluminum.

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8. The microtitre plate as set forth in claim 4, wherein the coating is reflective aluminum.

1 9. In a method of conducting a photon emitting
2 immunoassay in a multi-well microtitre plate, wherein an
3 antigen-antibody complex is formed in the wells and photons
4 emitted from the wells are detected by a photometric device,
5 the improvement comprising:

6 conducting the immunoassay in a microtitre
7 plate having a reflective metallized surface on the inner
8 surface of the bottom walls, and optionally on the inner
9 surface of the side walls, of the wells to reflect photons
10 emitted from the wells into the photometric device.

11 10. The method as set forth in claim 9, wherein the
12 reflective surface is a reflective metallized coating on the
13 inner surface of the well walls, said coating being selected
14 from the group consisting of reflective aluminum, tin,
15 magnesium, zinc, cadmium, indium, transition metals,
16 ceramics and semiconductors.

17 11. The method as set forth in claim 10, wherein the
18 reflective surface is an aluminum reflective surface on the
19 inner surface of the well walls.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 94/00158A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 B01L3/00 G01N21/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 04482 (PARK PHARMACEUTICALS) 4 April 1991 see page 1, line 3 - line 12; claims 8-10 see page 7, line 10 - page 8, line 16	1-3,9,12
Y	see page 14, line 17 - line 27 see page 14, line 9 - line 16; figure 6 --- -/--	5-7,10, 11,13,14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *I* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

6 June 1994

Date of mailing of the international search report

22.06.94

Name and mailing address of the ISA

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Authorized officer

Hocquet, A

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/CA 94/00158

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Week 8718, Derwent Publications Ltd., London, GB; AN 87-125114 & JP,A,62 066 141 (SUMITOMO) 25 March 1987 see abstract & PATENT ABSTRACTS OF JAPAN vol. 11, no. 261 (P-609) (2708) 25 August 1987 & JP,A,62 066 141 (SUMITOMO) 25 March 1987 see abstract ---	5-7,10, 11,13,14
X	DATABASE WPI Week 8620, Derwent Publications Ltd., London, GB; AN 86-129702 & SE,A,8 404 141 (WALLAC OY) 21 February 1986 see abstract ---	1,4,8,12
A	WO,A,89 12838 (WALLAC OY) 28 December 1989 see page 3, paragraph 3; figure 2 ---	1,12
A	see page 4, line 11 - line 16 ---	9
P,X	DATABASE WPI Week 9336, Derwent Publications Ltd., London, GB; AN 93-285786 & JP,A,5 203 562 (HITACHI) 10 August 1993 see abstract & PATENT ABSTRACTS OF JAPAN vol. 017, no. 626 (P-1647) 18 November 1993 & JP,A,05 203 562 (HITACHI) 10 August 1993 see abstract ---	1,12
E	US,A,5 298 753 (WALLAC OY) 24 March 1994 ---	1,9
E	EP,A,0 589 691 (WALLAC OY) 30 March 1994 see page 2, line 36 - line 52 see page 3, line 15 - line 25; figures -----	1,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9104482	04-04-91	US-A- 5082628 EP-A- 0491880	21-01-92 01-07-92
WO-A-8912838	28-12-89	EP-A,B 0380613 JP-T- 3500211 US-A- 5061853	08-08-90 17-01-91 29-10-91
US-A-5298753	29-03-94	NONE	
EP-A-0589691	30-03-94	NONE	